JOURNAL OF CLINICAL MICROBIOLOGY, July 1987, p. 1191–1194
0095-1137/87/071191-04$02.00/0

Vol. 25, No. 7

DNA Relatedness, Taxonomy, and Medical Significance of *Geotrichum capitatum*

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Received 10 December 1986/Accepted 30 March 1987

Among the clinical isolates hitherto identified as *Geotrichum capitatum*, two groups were defined from DNA-DNA reassociation experiments. This confirms the existence of two closely related, human-pathogenic *Geotrichum* species, namely, *G. capitatum* and *G. clavatum*. A third group of strains from cactus rots, though morphologically identical to *G. capitatum*, has a lower moles percent G+C of DNA. The three groups can be recognized by a combination of morphological and physiological characters.

Geotrichum capitatum (Diddens and Lodder) von Arx is a filamentous yeast which is occasionally encountered in clinical laboratories. It is mostly isolated from human sputum, frequently in association with lung disorders (7, 16). It also occurs as a secondary invader in debilitated hosts or patients receiving immunosuppressive treatment. It has been reported from cases of endocarditis (1, 28), sepsis (2, 17), and encephalitis (25). The species is thermotolerant, having an optimum development at temperatures above 30°C. *G. capitatum* was found to be pathogenic after experimental inoculation in mice (6) and rabbits (8). It is occasionally encountered in the digestive tract of various warmblooded animals other than humans (19). It was incriminated as a cause of abortion in a cow (10) and a horse (12).

In medical literature the species is mostly refered to as *Trichosporon capitatum* Diddens and Lodder, because it has conidia ("blastospores") produced from sympodially elongating cells, in addition to arthric conidia. However, the presence or absence of such conidia is usable as a taxonomic criterion at the species level only. In some species of *Trichosporon* this phenomenon is even strain dependent, and the conidial abundance can be influenced by cultural conditions. Morphologically similar species are known to belong to different classes of fungi. In modern taxonomy, the genus *Trichosporon* Behrend is conceived as a taxon of basidiomycetous affinity (27). *T. capitatum* does not stain with diazonium blue B and does not utilize urea or nitrate. These properties are indicative for an ascomycete (endomycete) rather than a basidiomycete relationship, since only a very few endomycetes are positive for these compounds.

The presence of micropores in the septa (22) points to a membership of the subclass *Endomycetes*. The discovery of a *Dipodascus* teleomorph in *T. capitatum* (3) confirms that the earlier recombination in the endomycetous genus *Geotrichum* Link:Fr. by von Arx et al. (24) was justified.

A closer examination of a series of mainly clinical isolates, provisionally identified as *G. capitatum*, revealed that several strains lacked some important key features. Four strains were able to grow with cellobiose, salicin, and arbutin, in contrast to the remaining *G. capitatum* strains. In addition, two of the atypical strains lacked the characteristic sympodial rachides; hence, they were monomorphic, producing arthric conidia only. Two strains originating from cactus rots were morphologically very close to *G. capitatum*. A study of the DNA of the respective strains was undertaken to establish whether the differences found have any taxonomic significance.

MATERIALS AND METHODS

Strains analyzed in the present study. The strains studied were: CBS 197.35, mating type A, authentic culture of *Dipodascus capitatus* de Hoog et al., ex woodpulp, E. Rennerfelt (3); CBS 425.71, an authentic culture of *Geotrichum clavatum* de Hoog et al., ex human lung tissue, D. G. Ahearn (3); CBS 571.82 = ATCC 10663, an authentic culture of *T. capitatum* Diddens and Lodder, ex wood pulp, E. Rennerfelt (4); CBS 576.82, ex human patient with asthma, N. G. M. Orie; CBS 578.82 = ATCC 28576, mating type **a**, ex sputum, L. do Carmo Sousa; CBS 207.83 = ATCC 46132, an authentic culture of *Blastoschizomyces pseudotrichosporon* Salkin et al., ex sputum, I. F. Salkin (20); CBS 598.83 = IP 1554-84, ex oral infection of human patient with leukemia (17); CBS 716.84 = IP 1630-86, ex digestive tract of swine, H. Saëz (19); CBS 758.85, an authentic culture of *Dipodascus spicifer* de Hoog et al., ex cactus rot, M. A. Lachance (3); CBS 327.86 = IP 1640-86, ex blood culture from human patient with leukemia, York, Pa., J. P. Manzella; and CBS 328.86 = IP 1556-84, ex blood culture of human patient with leukemia, E. Drouhet (29).

Media used for morphological study were 2% malt extract and 4% malt extract with 0.5% yeast extract. Slides were made in 0.9% NaCl. Assimilation and fermentation abilities were tested three times by the methods standardized by Van Der Walt and Yarrow (23).

DNA was isolated and purified by methods described by Price et al. (18), modified by Guého et al. (9). Samples were dialyzed against 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) and stored in a freezer. Ratios of $A_{260}/A_{280} = 1.85$ and $A_{230}/A_{260} \leq 0.5$ were used to determine the quality of preparations.

Nuclear DNA base composition, expressed as moles percent guanine plus cytosine, was determined from thermal denaturation profiles of the DNA by the method of Marmur and Doty (14). The curves were obtained on a Gilford 2400S recording spectrophotometer equipped with a thermoprogrammer. DNA of *Candida lusitaniae* (ATCC 42720) with T_m at 87.8°C in 1× SSC was employed as control standard. The resulting thermal denaturation curves were derived by the

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TABLE 1. Characteristics of DNA of 11 strains related to *G. capitatum*

Group	Strain	mol% G+C of total DNA ± SD[a]	mol% G+C of main peak ± SD[a]
I. *G. capitatum s.s.* (rachides +, cellobiose −, salicin −, arbutin −)	CBS 197.35	36.3 ± 0.18	40.2 ± 0.15
	CBS 571.82	33.7 ± 0.15	40.1 ± 0.32
	CBS 578.82	36.1 ± 0.34	40.5 ± 0.45
	CBS 207.83	37.1 ± 0.20	41.1 ± 0.07
	CBS 598.83	34.2 ± 0.14	39.9 ± 0.18
	CBS 716.84	36.2 ± 0.07	41.2 ± 0.15
	CBS 327.86	32.6 ± 0.05	39.7 ± 0.46
	CBS 328.86	32.3 ± 0.18	39.9 ± 0.57
II. *G. clavatum* (rachides −, cellobiose +, salicin +, arbutin +)	CBS 425.71	31.1 ± 0.02	40.0 ± 0.14
	CBS 576.82	33.2 ± 0.23	41.0 ± 0.02
III. *D. spicifer* (rachides +, cellobiose +, salicin +, arbutin +)	CBS 758.85	29.5 ± 0.38	38.7 ± 0.46

[a] SD, Standard deviation calculated from at least three thermal denaturation curves.

method of E. Guého (manuscript in preparation), by which the DNA was found to consist of two types, probably nuclear and mitochondrial. In the present study only the highest G+C values are given, calculated from main peaks of (nuclear) DNA, in addition to values obtained from untreated T_m curves.

DNA-DNA reassociation experiments were performed by the spectrophotometer method, as described by Seidler and Mandel (21) and modified by Kurtzman et al. (13).

RESULTS

On the basis of morphology and physiology, three groups could be distinguished among the strains identified until recently as *G. capitatum*. These groups are distinguished by the presence or absence of sympodial rachides and by growth or no growth with cellobiose, salicin, and arbutin. The moles percent of G+C found in the course of the present study confirmed the segregation of a third group (III), combining sympodial rachides with growth on the mentioned compounds (Table 1). Strains of this group have a relatively low G+C content of DNA. With this character no distinction can be made between groups I and II.

Table 2 presents the results of a series of DNA-DNA reassociation experiments. Eight combinations, including all members of group I, resulted in reassociation values of 80 to 100%, clearly indicating the identity of these strains. A reassociation value of 95.3% was found with CBS 571.82 and CBS 716.84, which differ by 1.1 mol% in G+C content of DNA. The two mating partners of *D. capitatus* had a reassociation value of 82.9%. Members of group I, including the type strains of *B. pseudotrichosporon*, *D. capitatus*, and *G. capitatum*, can consequently be regarded to represent a single species, for the anamorph of which the same *Geotrichum capitatum* should be applied.

DNA-DNA reassociation experiments between members of groups I and II resulted in low to intermediate values, ranging from 0 to 35%. CBS 425.71 and CBS 576.82, originating from human lungs and bronchiae, were found to be identical, with a DNA-DNA reassociation value of 93%. Strain CBS 758.85 (group III), isolated from rotting cactus cladodes, showed relatively low reassociation values with all medical strains belonging to groups I and II.

DISCUSSION

All strains studied show slow expansion growth (13 to 20 mm in diameter on YPGA after 10 days at 21°C), have their optimum development at 30 to 37°C, and have a relatively low percent G+C of DNA. Nevertheless, the strains with these characters are markedly different in their morphology, physiology, and ecology. The results are consistent with the results of the percent DNA-DNA reassociation studies.

Strains of group I are unable to utilize cellobiose, salicin, or arbutin. All are characterized by having sympodial rachides on slender conidiogenous cells, which are in more or less verticillate arrangement on ascending hyphae (Fig. 1). The conidia are short cylindrical to slightly clavate, with a flat basal scar and a broadly rounded apex. Their shape distinguishes them from arthric conidia, which are truncate or, if inflated, rounded at both ends (Fig. 1). Thus, group I was confirmed to be homogenous, showing DNA-DNA reassociation values of over 81 mol%. Nearly all strains of group I originate from warm-blooded animals, particularly from human sputum. According to the CBS files, the strains from wood pulp, CBS 571.82 and CBS 197.35, grew at warm,

TABLE 2. DNA relatedness between 11 strains related to *G. capitatum*

Strain	DNA relatedness (% ± SD)			
	CBS 571.82	CBS 197.35	CBS 425.71	CBS 758.85
CBS 197.35 (*G. capitatum*)	80.8 ± 0.3			
CBS 578.82 (*G. capitatum*)		82.9 ± 3.9		
CBS 207.83 (*G. capitatum*)	98.8 ± 0.3	82.0 ± 5.5		
CBS 716.84 (*G. capitatum*)	95.3 ± 0.6			
CBS 598.83 (*G. capitatum*)	98.2 ± 0.3			
CBS 327.86 (*G. capitatum*)	100.0 ± 0.0		35.0 ± 2.5	40.9 ± 2.4
CBS 328.86 (*G. capitatum*)	98.0 ± 1.9		0.0 ± 0.0	
CBS 425.71 (*G. clavatum*)	32.1 ± 1.4			47.1 ± 1.2
CBS 576.82 (*G. clavatum*)	20.9 ± 1.8		93.4 ± 7.4	64.4 ± 3.3
CBS 758.85 (*D. spicifer*)	8.8 ± 1.3		47.1 ± 1.2	

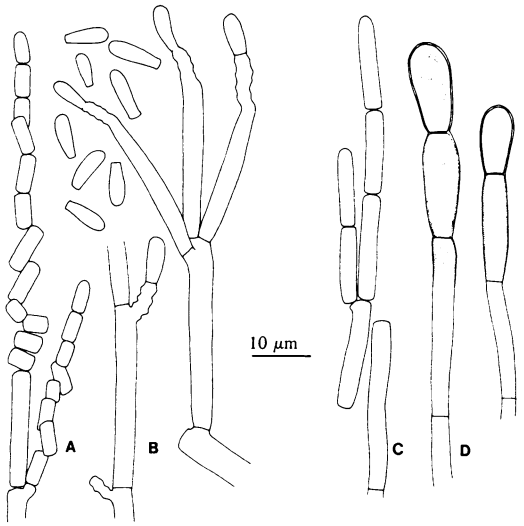

FIG. 1. Morphology of medical species of the *G. capitatum* complex. *G. capitatum*: (A) arthric conidia; (B) sympodial rachides with conidia. *G. clavatum*: (C) arthric conidia; (D) terminal swollen cells.

microaerobic conditions. The group includes an authentic strain of *G. capitatum*.

The strains that are able to utilize cellobiose, salicin, and arbutin are distributed over groups II and III. Group II, comprising strains from human patients, is characterized by an absence of sympodial rachides and by local presence of swollen hyphal ends. For this group the name *Geotrichum clavatum* is available (3). The single strain that constitutes group III, originating from cactus rot, has relatively large sympodial rachides and forms a *Dipodascus* teleomorph. It was recently described as *Dipodascus spicifer* (3).

Reassociation experiments of groups II and III with all strains of group I consistently gave low or relatively low values (Table 2), and the same was found after reassociation of the medical (II) with the nonmedical (III) strains. Reassociation of the DNAs of the two strains of group II confirmed their identity. The combinations of the single strain of group III with each strain of II gave intermediate values of 47.2 and 64.4%. This indicates nonidentity of the reassociated DNAs, but nevertheless might point to a close kinship, since such intermediate values have thus far not been found in reassociations of unrelated organisms.

G. capitatum in a broad sense can be easily distinguished from a second *Geotrichum* species that also is associated with human lung disorders, *G. candidum* Link:Fr. (5). *G. candidum*, the generic type species, has rapidly expanding colonies (about 80 mm in diameter in 10 days on YPGA at 21°C) and main hyphae of 7 to 12 μm which are often dichotomously branched at the colony margin, is unable to grow at 40°C, and does utilize D-glucitol. It is further distinguished from all medical strains of *G. capitatum* and *G. clavatum* by growing with D-xylose and is distinct from the anamorphs of *D. spicifer* in being unable to grow with cellobiose, salicin, and arbutin. The *Trichosporon beigelii* complex is markedly different from all *Geotrichum* species by mostly being able to utilize many more carbon compounds, e.g., L-arabinose, sucrose, maltose, α,α-trehalose, raffinose, ribitol, and D-glucitol.

All species mentioned, with the exception of *D. spicifer*, are common saprophytes of humans and other warm-blooded animals, either on the skin, in the respiratory tract, or in the intestines (e.g., see references 11 and 19). Most of the hosts have developed antibodies against these fungi (15). This explains the rarity of *Geotrichum* and *Trichosporon* infections, which may occur only when the patient has a suppressed immune system. Infections caused by *T. beigelii* are more frequent (26), and those with *G. candidum* are the rarest.

ACKNOWLEDGMENTS

We are much obliged to E. Drouhet, M.-A. Lachance, J. P. Manzella, O. Oelz, H. Saëz, and I. Salkin for supplying strains.

This work was performed with Georgia State University grant 86-034 to E.G.

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